



## **Pulse exposure of silver nanoparticles in acute and chronic toxicity tests with *D. magna***

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# Pulse exposure of silver nanoparticles in acute and chronic toxicity tests with *D. magna*

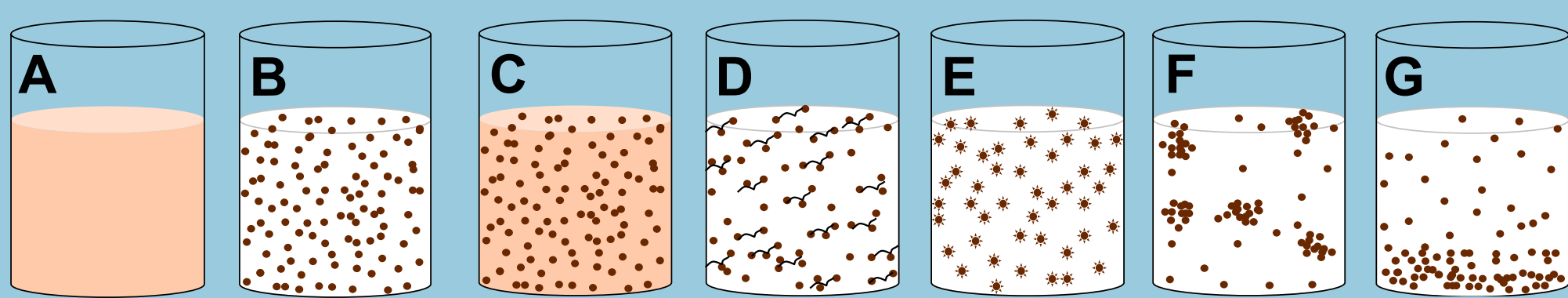
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## Introduction & objectives

Nanoparticles in suspension (B) behave differently than soluble chemicals (A) and may undergo dissolution (C), interact with media components (D), be submitted to surface/coating alterations (E), aggregation (F) and sedimentation (G). These processes challenge our attempt to control or even describe the exposure concentration and characteristics of NPs during ecotoxicity testing (Sørensen et al., Integrated Environmental Assessment and Management *in press*).



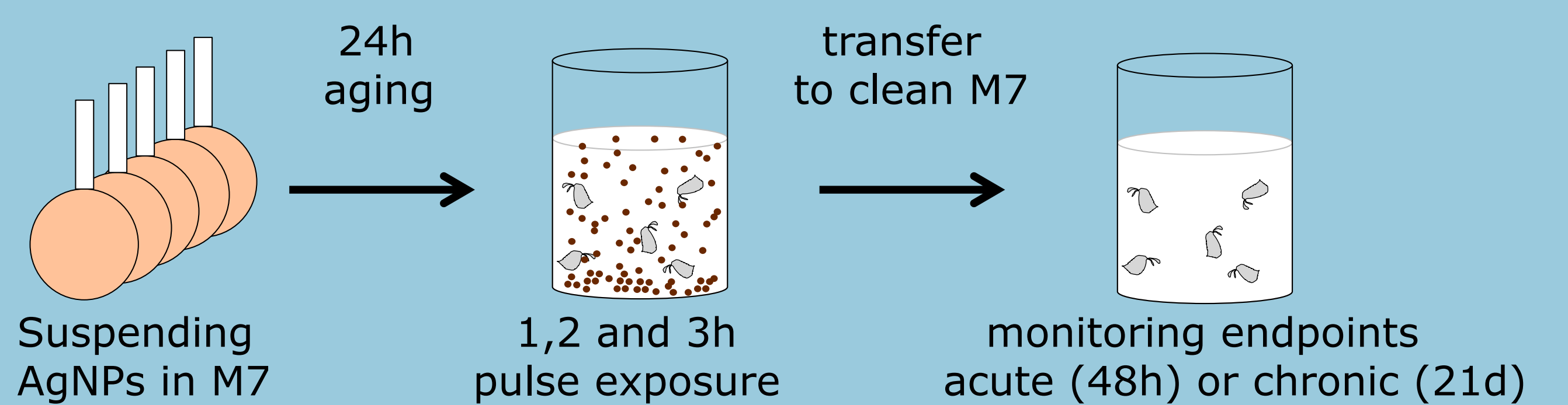
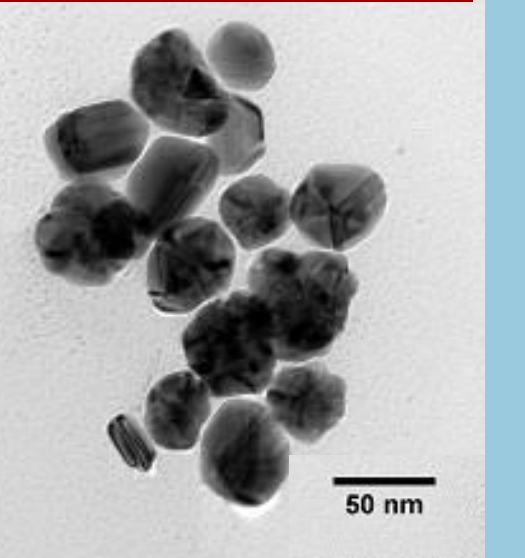
For silver nanoparticles (AgNPs), the use of aged test suspensions in a short-term (2h) algal test generated reproducible concentration-response data (Sørensen & Baun 2015, Nanotoxicology), indicating stable exposure concentrations. Here, we explore the same approach for another standard ecotoxicity test.

**The aim** is to investigate the applicability of a short-term (1-3h) pulse exposure to disclose acute and chronic effects of aged AgNP suspensions and dissolved silver (AgNO<sub>3</sub>) in *Daphnia magna*.

## Materials & Methods

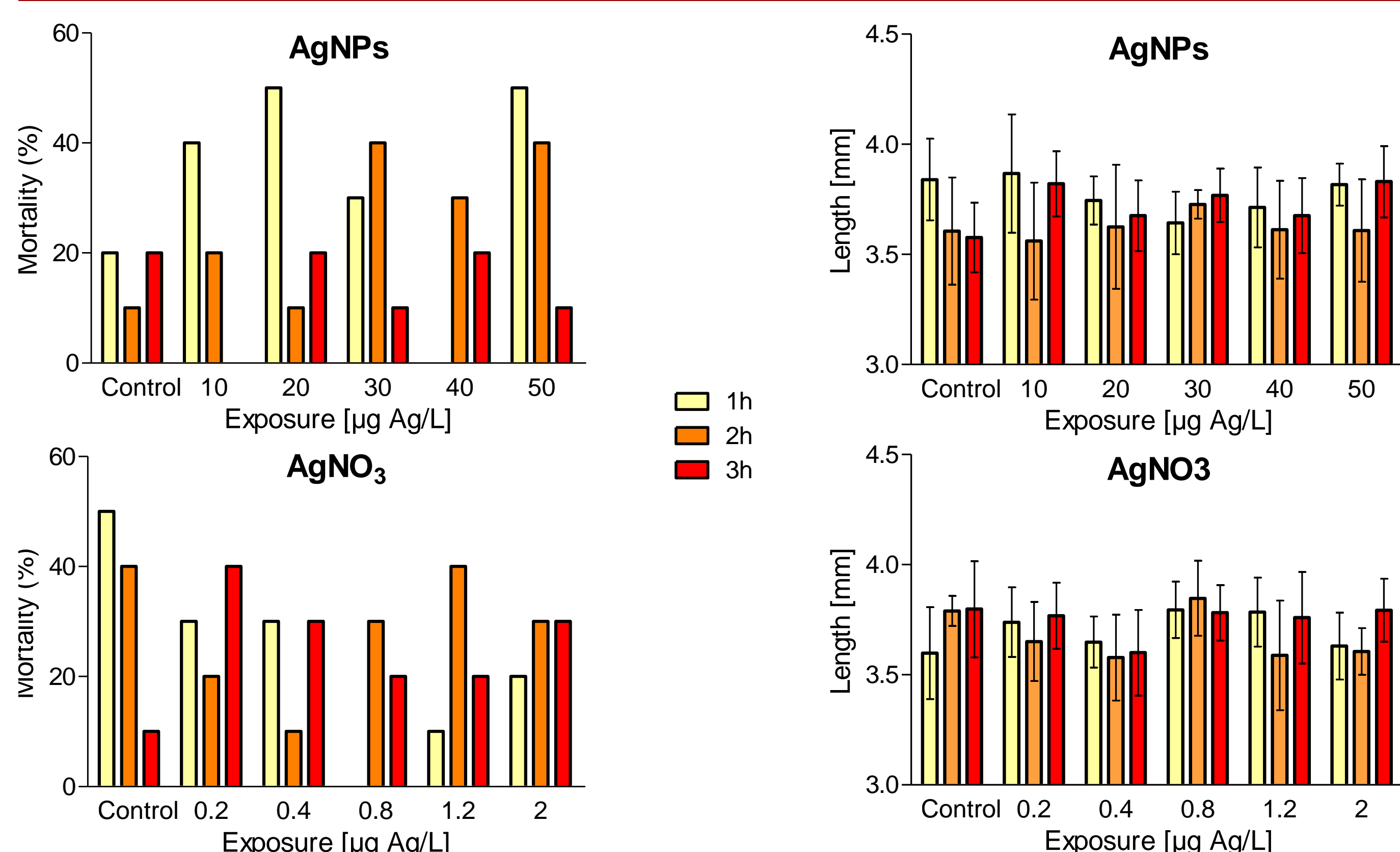
**Tested NP Reference** Citrate stabilized AgNPs nominal size 30 nm  
Dissolved silver (AgNO<sub>3</sub>)

**Medium Organism** Elenkt M7 (OECD 211)  
*Daphnia magna* neonates (<24h old)

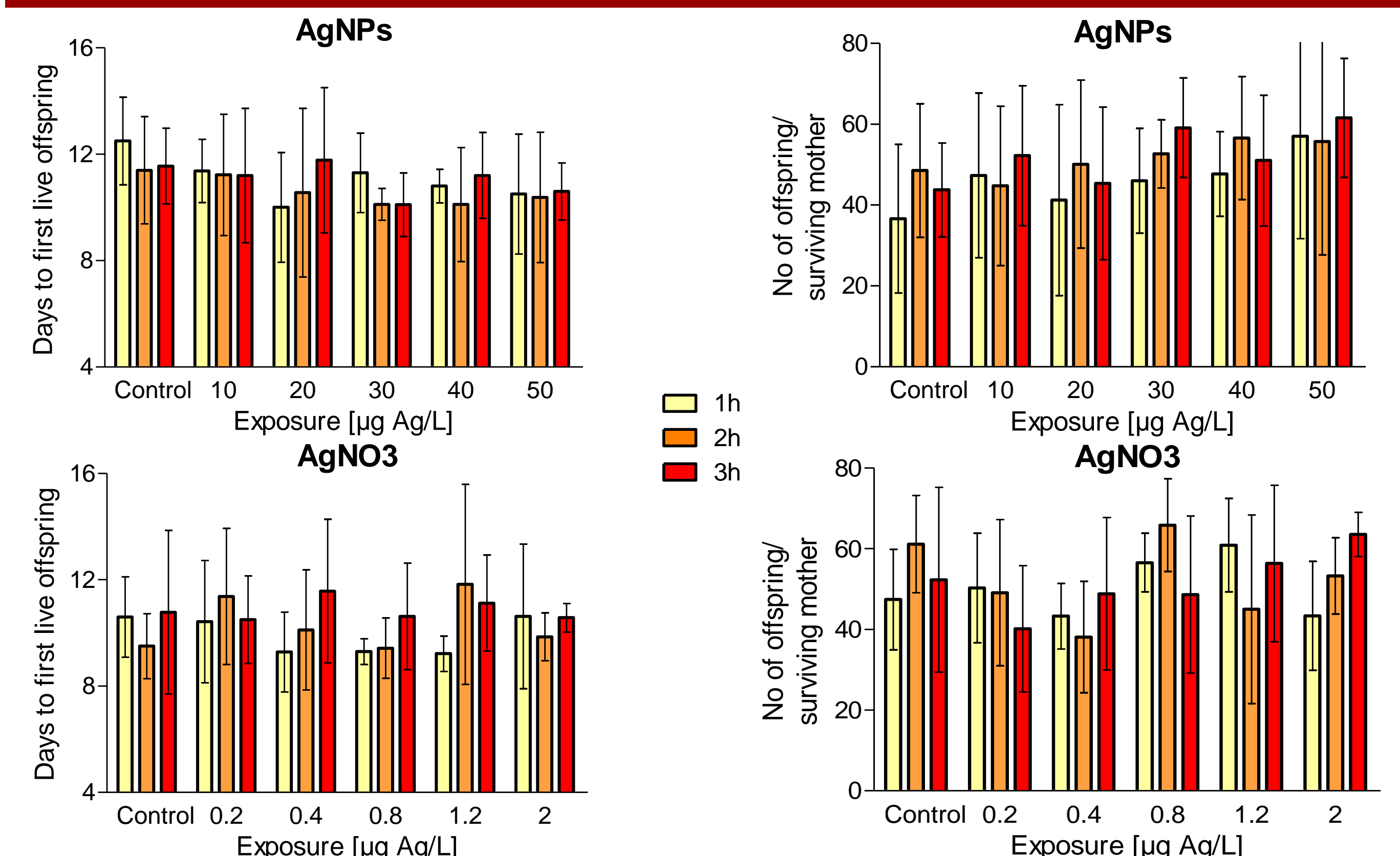


**Acute endpoint (48h)** Immobilization (according to OECD 202)  
**Chronic endpoints (21d)** Mortality, growth and reproduction

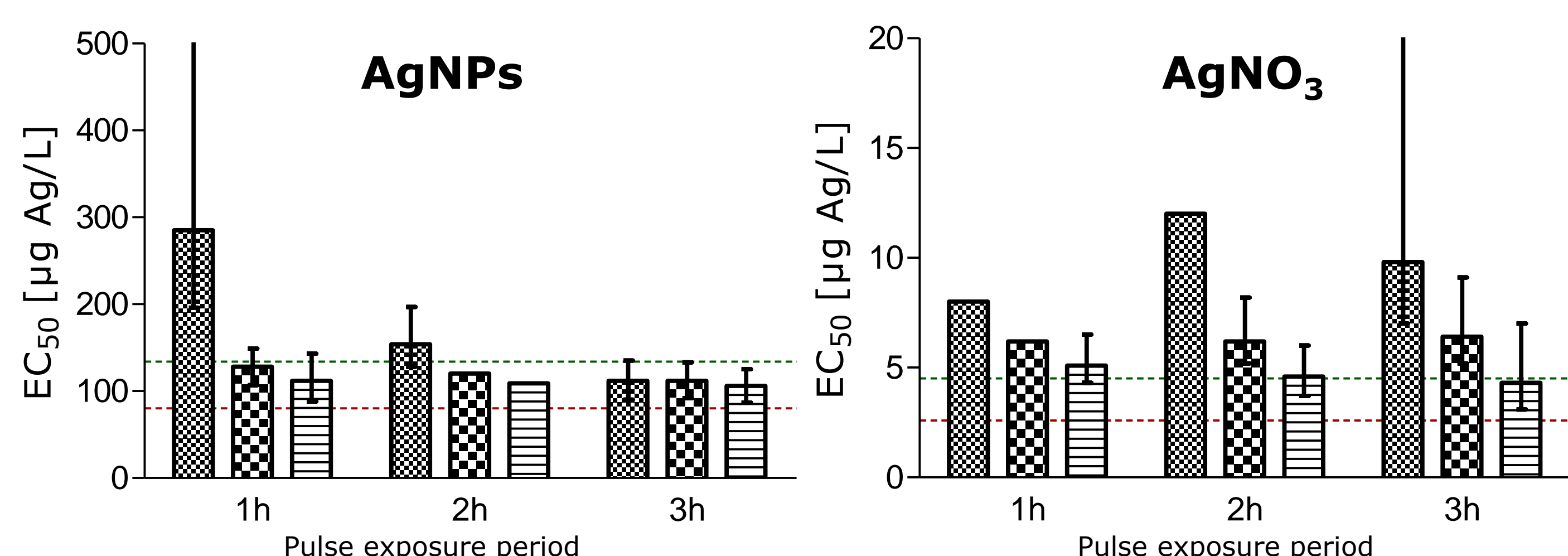
## Chronic endpoints (mortality & growth)



## Chronic endpoints (reproduction)



## Acute endpoint (Immobilization)



EC<sub>50,24h</sub> [μgAg/L] for continuous exposures (lines in graphs):

	AgNPs	AgNO <sub>3</sub>
Freshly made	134 [120;149]	4.5 [4.0;5.2]
Aged	< 80	2.6 [-]

0h post exposure  
24h post exposure  
48h post exposure

## Discussion & conclusion

### ACUTE ENDPOINTS

- The aging prior to testing increases toxicity of AgNPs and AgNO<sub>3</sub>, indicating that ions play a role
- The 24-48h immobility from 1-3h pulses of aged AgNPs are similar to that of 24h continuous exposure to freshly suspended AgNPs – i.e. this pulse setup is as sensitive as the continuous test but the short exposure makes monitoring and characterization of AgNPs during testing more feasible
- The toxicity generally increases slightly with pulse duration (1-3h) – this trend is less pronounced for AgNPs than AgNO<sub>3</sub> – i.e. 1h pulse can be applied in stead of 3h

### CHRONIC ENDPOINTS

- Mortality decreases with pulse duration for AgNPs – not for AgNO<sub>3</sub>. The double transfer of daphnids in short time may cause stress. High AgNO<sub>3</sub> control mortality (1-2h), so only 3h is considered
- No trends in growth between exposures
- Reproduction overall: No trend for pulse duration
- A very slight tendency for daphnids to reproduce sooner and have more offspring with AgNP-conc. Linear regression (pooled data 1-3h) confirms this (slope ≠ 0, P=0.04), but only the 50 μg/L (No of offspring) and 30 μg/L (Days to offspring) differs significantly from controls (P<0.05) by oneway ANOVA. For AgNO<sub>3</sub> (3h pulse) the same trend is observed for No of offspring
- The stimulation in reproduction may result from silver's antibacterial effect: Higher concentrations and/or other endpoints may be further tested

